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<b>(21) International Application Number:</b> PCT/EP98/02884 <b>(22) International Filing Date:</b> 15 May 1998 (15.05.98)  <b>(30) Priority Data:</b> 97401196.7 30 May 1997 (30.05.97) EP <b>(34) Countries for which the regional or international application was filed:</b> FR et al.  <b>(71) Applicant (for all designated States except US):</b> SYNTHÉLABO [FR/FR]; 22, avenue Galilée, F-92350 Le Plessis Robinson (FR).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> RENARD, Stéphane [FR/FR]; 290, avenue Napoléon Bonaparte, F-92500 Rueil Malmaison (FR). BESNARD, François [FR/FR]; 5, rue du Colonel Fabien, F-78220 Viroflay (FR). GRAHAM, David [IE/FR]; 14, côte de la Jonchère, F-78380 Bougival (FR).  <b>(74) Agent:</b> THOURET-LEMAITRE, Elisabeth; Synthélabo, 22, avenue Galilée, F-92350 Le Plessis Robinson (FR).			<b>(81) Designated States:</b> JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> SODIUM CHANNEL RECEPTOR			
<b>(57) Abstract</b>  hSLNAC1 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing hSLNAC1 polypeptides and polynucleotides in the design of protocols for the treatment of certain diseases and diagnostic assays for such conditions.			

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## SODIUM CHANNEL RECEPTOR

### FIELD OF INVENTION

This invention relates to newly identified  
5 polynucleotides, polypeptides encoded by them and to the use  
of such polynucleotides and polypeptides, and to their  
production. More particularly, the polypeptides of the  
present invention is a sodium channel receptor, hereinafter  
referred to as hSLNAC1.

10

### BACKGROUND OF THE INVENTION

The mdeg and epithelial sodium channel family is a  
recent family of proteins composed of the homomeric or  
15 multimeric assembly of two transmembrane domain polypeptides  
to form a sodium channel (Renard S. et al (1994) J. Biol.  
Chem. 269/17 pp12981-12986 ; Lingueglia E. et al (1994) J.  
Biol. Chem. 269/19, 13736-13739 ; Renard S., et al (1995)  
Pflügers Archiv- Eur. J. Physiol 430,299-307 ; Lingueglia et  
20 al (1995) Nature 378, 730-733 ; Waldmann et al (1996) J.  
Biol. Chem. 271, 10433-10436). Members of this family have  
been described as sodium channels or putative  
mechanosensitive channels, in numerous tissues from nematode  
to man. The presence of a large extracellular domain in this  
25 protein class suggests that they may play the role of a  
receptor for some endogenous transmitters. Opening of the  
channel may be linked to this receptor function.  
Pharmacological properties of such a receptor are still  
largely unknown, but the diuretic amiloride is known to  
30 block most sodium channels of this family. Agonists or  
antagonists may be used, for example, to treat neuronal  
degenerescence problems, hyperalgesia, Alzheimer disease,  
Parkinson disease, chorea, muscular spasm, epilepsy, stroke,  
cardiac diseases, schizophrenia, depression, nicotine  
35 dependence, morphine dependence, amyotrophic lateral  
sclerosis, multiple sclerosis, inflammation, pain, cancer,  
obesity, to mimic or antagonize effect of some endogenous  
transmitter peptides in the central or peripheral nervous  
system, such as for instance opioids or anti-opioids, to  
40 alter gustative perception, to cause analgesia or  
anesthesia, or to diagnose or treat any disorder related to  
abnormal expression of this protein.

### SUMMARY OF THE INVENTION

45 The present inventors have found a new class of sodium  
channel protein. This new subunit may be responsible for  
some nervous system transmissions, disorders or may be a  
target to regulate some transmissions linked to various  
pathologies.

In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is a sodium channel, as well as fragments, analogs or derivatives. The polypeptide of the present invention is of human origin.

5 In accordance with another aspect of the present invention, there are provided polynucleotides which encode such polypeptide.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such  
10 polypeptide by recombinant techniques.

In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with hSLNAC1 imbalance with said identified  
15 compounds.

Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate hSLNAC1 activity or levels.

These and other aspects of the present invention should  
20 be apparent to those skilled in the art from the teachings herein.

#### **Brief Description of the Figures**

The following drawings are illustrative of the embodiments of the invention and are not meant to limit the  
25 scope of the invention as encompassed by the claims.

**Figure 1** illustrates the phylogenetic relationship between hSLNAC1 and other members of this sodium channel family. The length of each pair of branches represents the distance between sequence pairs. Alignment was performed with the  
30 clustal algorith of MEGALIGN (version 3.10a ) from DNASTAR.

**Figure 2** shows an aminoacid sequence comparison of the hSLNAC1 receptor with other members of the sodium channel family from the phylogenetic tree of Figure 1. The alignment was performed with the clustal algorith of MEGALIGN (version  
35 3.10a ) from DNASTAR. Regions of homology to SLNAC1 are shaded in black.

In Figures 1 and 2, the terms used have the following meanings :

hNACHA : alpha subunit of epithelial sodium channel  
40 (accession SW SCAA\_HUMAN)

hNACHB : beta subunit of epithelial sodium channel

(accession PIR I38203)

hNACHC : gamma subunit of epithelial sodium channel  
(accession PIR I38204)

hNACHD : delta subunit of sodium channel (accession PIR  
I39196)

- 5 ASIC : ASIC sodium channel (as published in Nature 368, 173-  
177- accession RNU94403)

MDEG : MDEG sodium channel (accession gi 1280439)

HAFANACH : sodium channel protein from aplysia, gated by  
FRMF-amide (accession gi 1149511).

- 10 **Figure 3** illustrates secondary structural features of this  
hSLNAC1 protein with the hydrophilicity, hydrophobicity, the  
propensity to generate alpha helix, beta sheet, turn or coiled  
regions, the propensity to be on surface of the protein, and  
the flexible regions. The boxed areas are the areas which  
15 correspond to the regions indicated. The hydrophobicity plot  
illustrates hydrophobic areas of the protein sequence which  
are in the lipid bilayer and hydrophilic areas which are  
outside the lipid bilayer. The antigenicity of the protein  
fragments is higher in areas exposed to the surface, which  
20 are hydrophilic and flexible regions. The analysis was  
performed with Protean (version 3.08a) from DNASTAR.

#### DESCRIPTION OF THE INVENTION

##### Definitions

25

The following definitions are provided to facilitate  
understanding of certain terms used frequently herein.

- 30 "Receptor Activity" or "Biological Activity of the  
Receptor" refers to the metabolic or physiologic function of  
said hSLNAC1 including similar activities or improved  
activities or these activities with decreased undesirable  
side-effects. Also included are antigenic and immunogenic  
activities of said hSLNAC1.

35

"hSLNAC1 polypeptide" refers among others to a polypeptide  
comprising the amino acid sequence set forth in SEQ ID NO:2  
or an allelic variant thereof.

- 40 "hSLNAC1 gene" or "hSLNAC1 polynucleotide" refer to a  
polynucleotide comprising the nucleotide sequence set forth  
in SEQ ID NO:1 or allelic variants thereof and/or their  
complements.

45

"Antibodies" as used herein includes polyclonal and

monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

5 "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide  
10 or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any  
15 polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA,  
20 and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions  
25 comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases  
30 such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and  
35 cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by  
40 peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than  
45 the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in  
50 more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a

- polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods.
- Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.
- "Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence

by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., et al., *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO : 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO : 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number



of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of Figure 3 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO : 1. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

### 30 Polypeptides of the Invention

In one aspect, the present invention relates to hSLNAC1 polypeptides. The hSLNAC1 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Also included within hSLNAC1 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. hSLNAC1 polypeptide comprising an amino acid sequence having at least 80% identity, preferably at least 90% identity, and even still more preferably at least 95% to the amino acid sequence encoded by the cDNA contained in

ATCC 97987 are also included within the present invention. Preferably hSLNAC1 polypeptides exhibit at least one biological activity of the receptor.

5 The hSLNAC1 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an  
10 additional sequence for stability during recombinant production.

Fragments of the hSLNAC1 polypeptides are also included in the invention. A fragment is a polypeptide having an  
15 amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned hSLNAC1 polypeptides. As with hSLNAC1 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most  
20 preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of hSLNAC1 polypeptide. In this context "about" includes the  
25 particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation  
30 polypeptides having the amino acid sequence of hSLNAC1 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including  
35 the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil  
40 and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments.  
45 Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

50

Preferably, all of these polypeptide fragments retain

the biological activity of the receptor, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions i.e., those that substitute a residue  
5 with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants  
10 in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The hSLNAC1 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include  
15 isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

20

#### Polynucleotides of the Invention

Another aspect of the invention relates to hSLNAC1  
25 polynucleotides. hSLNAC1 polynucleotides include isolated polynucleotides which encode the hSLNAC1 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, hSLNAC1 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence  
30 set forth in SEQ ID NO:1 encoding a hSLNAC1 polypeptide of SEQ ID NO : 2, and polynucleotide having the particular sequence of SEQ ID NO:1.

hSLNAC1 polynucleotides further include a  
35 polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the hSLNAC1 polypeptide of SEQ ID NO : 2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO : 1 over its entire length. In this  
40 regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most  
45 preferred. Also included under hSLNAC1 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 or contained in the cDNA insert in the plasmid deposited with the ATCC  
50 Deposit number 97987 (see herein-below) to hybridize under conditions useable for amplification or for use as a probe or marker.

Such a sequence may, for example, consists in the nucleotide sequence SEQ ID NO: 3 which constitutes another object of the present invention, as well as the its deduced polypeptide sequence SEQ ID NO: 4 and polynucleotides and polypeptide sequences having at least 80% identity, preferably 90% identity, more preferably 97 % identity and still more preferably at least 99% identity to said sequence SEQ ID NO: 3 and SEQ ID NO: 4. The cDNA deposited at the ATCC with Deposit Number 97987 is identical to SEQ ID NO: 3. SEQ ID NO: 3 may be used as probe for diagnostic.

So, according to the present invention, hSLNAC1 polynucleotide include a nucleotide sequence having at least 80% identity to the cDNA insert deposited at the ATCC with Deposit Number 97987, and a nucleotide sequence comprising at least 15 contiguous nucleotides of such cDNA insert. The invention also provides polynucleotides which are complementary to all the above hSLNAC1 polynucleotides.

A deposit containing a hSLNAC1 cDNA has been deposited with the American Type Culture Collection (ATCC), 12301 Park Lawn Drive, Rockville, Maryland 20852, USA, on April 16, 1997, and assigned ATCC Deposit Number 97987. The deposited material (clone) is plasmid pCDNA3 which can be obtained from Invitrogen, Inc. containing that further contains the full length hSLNAC1 cDNA, referred to as p3SLNAC1 upon deposit. The cDNA insert is within KpnI-NotI sites in the vector. The nucleotide sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit has been made under the terms of the Budapest Treaty on the international recognition of the deposit of micro-organisms for purposes of patent procedure.

hSLNAC1 of the invention is structurally related to other proteins of the sodium channel family, as shown by the results of sequencing the cDNA of SEQ ID NO:1. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 76 to 1629) encoding a polypeptide of 518 amino acids of SEQ ID NO:2.

Amino acid sequence of SEQ ID NO:2 has about 52,1% identity and 71,8% similarity (using GCG gap algorithm) in 518 amino acid residues with ASIC Protein (gi/2039366) Waldmann R. et al., *Nature*, (1997) 386:173-177}. Nucleotide sequence of SEQ ID NO:1 has about 60,7% identity (using GCG gap algorithm) in 1554 nucleotide residues with ASIC Protein (gi/2039365).

One polynucleotide of the present invention encoding hSLNAC1 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human cerebellum using the expressed sequence tag (EST) analysis (Adams, M.D., et al. *Science* (1991) 252:1651-1656; Adams, M.D. et al., *Nature*, (1992) 355:632-634; Adams, M.D., et al., *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding hSLNAC1 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 (nucleotide number 13 to 1641), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of hSLNAC1 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding hSLNAC1 variants comprising the amino acid sequence of hSLNAC1 polypeptide of SEQ ID NO : 2 in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof, or to the cDNA insert in the plasmid deposited at the ATCC with Deposit Number 97987 or a  
5 fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding hSLNAC1 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the hSLNAC1 gene. Such hybridization  
10 techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at  
15 least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding  
20 hSLNAC1 polypeptide comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO : 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization  
25 techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x  
30 Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present  
35 invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

#### **Vectors and Host Cells**

40

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors; and the production of hSLNAC1  
45 polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a  
50 calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged

in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, zeocin, hygromycin, or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate heterologous hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion

signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson et al., *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The hSLNAC1 receptor can be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention



may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

### Diagnostic Assays

5        This invention also relates to the use of hSLNAC1 polynucleotides for use as diagnostic reagents. Detection of a mutated form of hSLNAC1 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a  
10       disease which results from under-expression, over-expression or altered expression of hSLNAC1. Individuals carrying mutations in the hSLNAC1 gene may be detected at the DNA level by a variety of techniques.

15       Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to  
20       analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled hSLNAC1 nucleotide sequences.  
25       Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by  
30       direct DNA sequencing. See, e.g., Myers et al., *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton et al., *Proc Natl Acad Sci USA* (1985) 85: 4397-  
35       4401. In another embodiment, an array of oligonucleotides probes comprising hSLNAC1 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to  
40       address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., *Science*, Vol 274, pp 610-613 (1996)).

45       The diagnostic assays offer a process for diagnosing or determining a susceptibility to the treatment of diseases through detection of mutation in the hSLNAC1 gene by the methods described. Said diseases are, in particular, chosen among the following : neuronal degenerescence problems,  
50       hyperalgesia, Alzheimer disease, Parkinson disease, chorea, muscular spasm, epilepsy, stroke, cardiac diseases,

schizophrenia, depression, nicotine dependence, morphine dependence, amyotrophic lateral sclerosis, multiple sclerosis, inflammation, pain, cancer, obesity, to mimic or antagonize effect of some endogenous transmitter peptides in the central or peripheral nervous system (such as for  
5 instance opioids or anti-opioids), alteration of gustative perception, disorder related to abnormal expression of the hSLNAC1 protein.

In addition, the same diseases can be diagnosed by  
10 methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of hSLNAC1 polypeptide or hSLNAC1 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of  
15 polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an hSLNAC1, in a sample derived from a host are well-known to those of skill in the art.  
20 Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

#### **Detection of hSLNAC1 Gene Expression**

25 The expression level of the hSLNAC1 gene can be readily assayed by one of ordinary skill in the art. By "assaying the expression level of the gene encoding the hSLNAC1 polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the hSLNAC1 polypeptide  
30 or the level of the mRNA encoding the hSLNAC1 polypeptide in a biological sample (e.g., by determining or estimating absolute protein level or mRNA level). By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or  
35 other source which contains hSLNAC1 polypeptide or hSLNAC1 mRNA. Such tissues include cerebellum, brain, midbrain, spinal cord, nerve endings, retina, breast, pituitary, heart, placenta, lung, skeletal muscle, kidney, and pancreas. Biological samples include mammalian tissues  
40 which contain hSLNAC1 polypeptide. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits, and humans. Particularly preferred are humans. Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-  
45 chloroform method described in Chomczynski and Sacchi (Anal. Biochem. 162:156-159 (1987)). Levels of mRNA encoding the hSLNAC1 receptor are then assayed using any appropriate method. These include Northern blot analysis (Harada et al., Cell 63:303-312 (1990)), S1 nuclease mapping (Harada et al., Cell 63:303-312 (1990)), the polymerase chain reaction  
50 (PCR), reverse transcription in combination with the

polymerase chain reaction (RT-PCR) (Fujita et al., *Cell* 49:35-36 (1990)), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

As discussed here-above, assaying hSLNAC1 polypeptide levels in a biological sample can occur using antibody-based techniques. For example, hSLNAC1 polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M. et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M. et al., *J. Cell. Biol.* 105: 3087-3096 (1987)). Other antibody-based methods useful for detecting hSLNAC1 polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), biotin and fluorescent labels, such as fluorescein and rhodamine.

## 20 **Chromosome Assays**

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease. In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a hSLNAC1 polypeptide gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance In Man, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease. Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## 20 Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the hSLNAC1 polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the hSLNAC1 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

Non-limiting examples of polypeptides or peptides that can

be used to generate hSLNAC1 polypeptide-specific antibodies include: a polypeptide comprising amino acid residues from about 75 to about 81 of SEQ ID NO 2 ; a polypeptide comprising amino acid residues from about 96 to about 106 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 130 to about 137 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 215 to about 234 of SEQ ID NO 2 ; a polypeptide comprising amino acid residues from about 240 to about 250 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 287 to about 297 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 302 to about 310 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 341 to about 352 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 394 to about 405 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 419 to about 430 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 471 to about 489 of SEQ ID NO 2; a polypeptide comprising amino acid residues from about 517 to about 525 of SEQ ID NO 2; and a polypeptide comprising amino acid residues from about 535 to about 545 of SEQ ID NO 2. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the hSLNAC1 polypeptide.

The above-mentioned antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against hSLNAC1 polypeptides may also be employed to treat, among others, patients with nervous system disorders, to treat neuronal degenerescence problems, Alzheimer disease, Parkinson disease, chorea, muscular spasm, epilepsy, stroke, cardiac diseases, schizophrenia, depression, nicotine dependence, morphine dependence, amyotrophic lateral sclerosis, inflammation, pain, multiple sclerosis, cancer, obesity, to mimic or antagonize effect of some endogenous transmitter peptides in the central or peripheral nervous system (such as for instance opioids or anti-opioids), to alter gustative perception, to cause analgesia or anesthesia, or to treat any disorder related to abnormal expression of said hSLNAC1 polypeptide.

### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with hSLNAC1 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from , among others, nervous system disorders, neuronal degenerescence

problems, Alzheimer disease, Parkinson disease, chorea, muscular spasm, epilepsy, stroke, cardiac diseases, schizophrenia, depression, nicotine dependence, morphine dependence, amyotrophic lateral sclerosis, inflammation, pain, multiple sclerosis, cancer, obesity, to mimic or  
5 antagonize effect of some endogenous transmitter peptides in the central or peripheral nervous system (such as for instance opioids or anti-opioids), to cause analgesia or anesthesia, or to treat any disorder related to abnormal expression said hSLNAC1 polypeptide.  
10 Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering hSLNAC1 polypeptide via a vector directing expression of hSLNAC1 polynucleotide in vivo in order to induce such an immunological response to produce antibody to  
15 protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological  
20 response in that mammal to a hSLNAC1 polypeptide wherein the composition comprises a hSLNAC1 polypeptide or hSLNAC1 gene. The vaccine formulation may further comprise a suitable carrier. Since hSLNAC1 polypeptide may be broken down in the stomach, it is preferably administered parenterally  
25 (including subcutaneous, intramuscular, intravenous or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the  
30 formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may  
35 be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the  
40 art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

#### Screening Assays

45

The hSLNAC1 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present  
50 invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and

ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan et al., *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

5 hSLNAC1 polypeptides may be responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate hSLNAC1 on the one hand and which can  
10 inhibit the function of hSLNAC1 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as, among others, nervous system disorders, to treat neuronal degenerescence problems, Alzheimer disease, Parkinson disease, chorea,  
15 muscular spasm, epilepsy, stroke, cardiac diseases, schizophrenia, depression, nicotine dependence, morphine dependence, amyotrophic lateral sclerosis, inflammation, pain, multiple sclerosis, cancer, obesity, to mimic or antagonize effect of some endogenous transmitter peptides in  
20 the central or peripheral nervous system (such as for instance opioids or anti-opioids), to alter gustative perception, to cause analgesia or anesthesia, or to treat any disorder related to abnormal expression of said hSLNAC1 polypeptide.

25 Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as, among others, nervous system disorders, to treat neuronal degenerescence problems, Alzheimer disease,  
30 Parkinson disease, chorea, muscular spasm, epilepsy, stroke, cardiac diseases, schizophrenia, depression, nicotine dependence, morphine dependence, amyotrophic lateral sclerosis, inflammation, pain, multiple sclerosis, cancer, obesity, to mimic or antagonize effect of some endogenous  
35 transmitter peptides in the central or peripheral nervous system (such as for instance opioids or anti-opioids), to alter gustative perception, to cause analgesia or anesthesia, or to treat any disorder related to abnormal expression of said hSLNAC1 polypeptide.

40 In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*.  
45 Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

50 The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor

is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using  
5 detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for  
10 conducting such screening assays are well understood in the art.

Alternatively, it is also possible to mutate the hSLNAC1 cDNA in order to produce a constitutively active  
15 sodium channel (Waldmann et al., J. Biol. Chem. 271 pp 10433-10436 ; Huang and Chalfie, Nature 367, 467-470). The mutation may for instance consists in changing glycine 411 to phenylalanine. Then, the constitutively active sodium channel may be expressed in host cells to produce a  
20 screening assay where sodium channel activity is permanent. The recording of channel activity may be carried out either by membrane voltage analysis, directly (patch clamp for example) or indirectly (fluorescent probes, cell death by measurement of lactate dehydrogenase release, for example),  
25 or by sodium entry measurement (radioactive sodium influx, fluorescent probes or reporter genes for example).

Examples of potential hSLNAC1 antagonists include antibodies or, in some cases, oligonucleotides or proteins  
30 which are closely related to the ligand of the hSLNAC1, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

35

#### **Prophylactic and Therapeutic Methods**

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient  
40 amounts of hSLNAC1 activity.

If the activity of hSLNAC1 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound  
45 (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the hSLNAC1, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

50

In another approach, soluble forms of hSLNAC1



polypeptides still capable of binding the ligand in competition with endogenous hSLNAC1 may be administered. Typical embodiments of such competitors comprise fragments of the hSLNAC1 polypeptide.

- 5 In still another approach, expression of the gene encoding endogenous hSLNAC1 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example,  
10 O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee et al., *Nucleic Acids Res* (1979) 6:3073; Cooney et al., *Science* (1988) 241:456; Dervan et al., *Science*  
15 (1991) 251:1360. These oligomers can be administered per se or the relevant oligomers can be expressed in vivo.

- For treating abnormal conditions related to an under-expression of hSLNAC1 and its activity, several approaches  
20 are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates hSLNAC1, i.e., an agonist as described above, in combination with a pharmaceutically acceptable  
25 carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of hSLNAC1 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective  
30 retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces  
35 infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic*  
40 *Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

#### 45 Formulation and Administration

- Peptides, such as the soluble form of hSLNAC1 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable  
50 pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or

compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art.

5 The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

10 Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the  
15 pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants  
20 such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

25 The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages,  
30 however, are in the range of 0.1-100  $\mu\text{g/kg}$  of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to  
35 require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

40 Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a  
45 polypeptide ex vivo, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

**Splice variants**

50

To ascertain the existence of splice variants of SEQ ID N°1 and 3, in some tissues of interest, Polymerase Chain Reactions were performed with oligonucleotides whose sequences were deduced from SEQ ID N°1. These nucleotides are : GGCGGCCGCTCTAGAACTAG ; GCTGCTGGCAAGAAACAAAG ;  
5 ATTTAACTCTGGCGCTGATGG ; GTCTAGGATCTCGAGGATGG ;  
ATGCTTCGCAAGGACTCGTG ; GAAAAGCTACGTGCAGGCTAG. The last pair of oligonucleotides allowed the amplification from dorsal root ganglia of a sequence differing in 79 nucleotides. These 79 nucleotides are inserted in the 3' part of the  
10 coding sequence and allowed the generation of SEQ ID N°5. Due to the reading frame change introduced by this insertion, the deduced protein sequence SEQ ID N°6 differs from SEQ ID N°4 in its C-terminal. As dorsal root ganglia are involved in painful perception, SEQ ID N°5 can be  
15 expected to encode a protein whose function is linked to algnesia or analgesia.

So according to another aspect, the invention also relates to an isolated polynucleotide comprising a nucleotide  
20 sequence that has at least 80%, preferably 90 %, more preferably 97 %, and still more preferably more than 99 % identity to a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:6 over its entire length; or a nucleotide sequence complementary to said nucleotide  
25 sequence.  
According to still another aspect, the invention also relates to polypeptide comprising an amino acid sequence which is at least 80%, preferably 90 %, more preferably 97 %, and still more preferably more than 99 %, identical to the amino acid sequence of SEQ ID NO:6 over its  
30 entire length.

### Examples

35 The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the  
40 invention.

#### Example 1 :

##### 45 Cloning the Human Sodium Channel

The sequence of the hSLNAC1 was first identified by searching a database containing approximately 1 million human ESTs, which was generated using high throughput  
50 automated DNA sequence analysis of randomly selected human cDNA clones (Adams, M.D. et al., Nature 377:3-174 (1995);

- Adams, M.D. et al., *Nature* 355:632-634 (1992); and Adams, M.D. et al., *Science* 252:1651-1656 (1991)). Sequence homology comparisons of each EST were performed against the GenBank database using the blastn and tblastn algorithms (Altschul, S.F. et al., *J. Mol. Biol.* 215:403-410 (1990)).
- 5 A specific homology search using the known human MDEG amino acid sequence against this human EST database revealed one EST (HGS826465), from a cerebellum cDNA library, with approximately 50% similarity to MDEG. HGS826465 contains 1710 bp and the sequence comparison suggested that it
- 10 contained the complete open reading frame of a new protein. Sequence of the gene was confirmed by double strand DNA sequencing using the TaqFs (Perkin Elmer) and the gene was shown to be completely new by a blast search against Genbank release 98. An expression vector construct was made by
- 15 inserting the KpnI-NotI fragment carrying the entire hSLNAC1 coding region into the KpnI-NotI site of the expression vector pCDNA3(-) (Invitrogen, Inc). This construct was named p3SLNAC1.

20

### Example 2

#### *Cloning and Expression of hSLNAC1 in Mammalian Cells*

- 25 A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements
- 30 include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and
- 35 the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as PSVL and PMSG (Pharmacia, Uppsala, Sweden),
- 40 pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109) and pCDNA3(-) (Invitrogen). Mammalian host cells that could be used include, human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster
- 45 ovary (CHO) cells.

- Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr,
- 50 gpt, neomycin, zeocin or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem. J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vector pcDNA3(-) contains the strong promoter (CMV) of the Cytomegalovirus. Multiple cloning sites, e.g., with the restriction enzyme cleavage sites KpnI, NotI, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the bovine growth hormone gene.

#### **Example 2(a): Cloning and Expression in COS Cells**

The expression plasmid, p3SLNAC1, is made by cloning a cDNA encoding hSLNAC1 into the expression vector pcDNA3(-) (which can be obtained from Invitrogen, Inc.). The expression vector pcDNA3(-) contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker; (5) a polyadenylation from the bovine growth hormone gene arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the polyadenylation signal by means of restriction sites in the polylinker. pcDNA3(-) contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the hSLNAC1 polypeptide is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The hSLNAC1 cDNA of the deposited clone is in the bluescript vector (Stratagene), it is excised from the bluescript vector with KpnI and Not I. The vector, pcDNA3(-), is digested with KpnI and Not I. The PCR amplified DNA fragment and the linearized vector are then ligated. The ligation mixture is transformed into *E. coli* strain DH5 $\alpha$  (available from GIBCO BRL), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid

DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the hSLNAC1-encoding fragment.

For expression of recombinant hSLNAC1 polypeptide, COS  
5 cells are transfected with an expression vector, as described above, using DEAE-Dextran, as described, for instance, in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under  
10 conditions for expression of hSLNAC1 by the vector.

**Example 2(b): Cloning and Expression in HEK293 Cells**

15 The vector p3SLNAC1 is used for the expression of hSLNAC1 protein. Plasmid p3SLNAC1 is described in example 1. The plasmid contains the mouse neomycin resistance gene under control of the SV40 early promoter. HEK293 Cells are transfected with these plasmids can be selected by growing  
20 the cells in a selective medium (containing 1 mg/ml Geneticin, Life Technologies). Cells grown in presence of 1mg/ml concentrations of Geneticin develop resistance to the drug by producing the target enzyme, Neomycin Resistance. If a second gene is linked to the Neomycin Resistance gene,  
25 it is usually co-expressed. It is known in the art that this approach may be used to develop cell lines expressing large amounts of the protein of interest, up to 1 pmole per mg of cell membrane in the case of a receptor. Subsequently, when the Geneticin is withdrawn, cell lines  
30 are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express hSLNAC1 in a  
35 regulated way in mammalian cells (Gossen, M. and Bujard, H., *Proc. Natl. Acad. Sci. USA* 89: 5547-5551(1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated  
40 into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin.

HEK293 cells are used for transfection. 20 µg of the  
45 expression plasmid p3SLNAC1 is transfected using calcium phosphate (Chen C, Okayama H, (1987) *Mol. Cell. Biol.* ; 7 : 2745-2752.). The plasmid pcDNA3(-) contains a dominant selectable marker, the neomycin resistance gene from Tn5 encoding an enzyme that confers resistance to a group of  
50 antibiotics including Geneticin. The cells are seeded in MEM supplemented with 1 mg/ml Geneticin. After 2 days, the cells

are trypsinized and seeded in cloning plates in MEM supplemented with 1 mg/ml Geneticin. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks. Clones growing are then transferred to new 6-well plates. Expression of the desired gene product is analyzed, for instance, by Northern blot.

### Example 3

#### 10. *Tissue distribution of hSLNAC1 mRNA expression*

Northern blot analysis can be carried out to examine hSLNAC1 gene expression in human tissues, using methods described by, among others, Sambrook et al., cited above. A cdNA probe containing the entire nucleotide sequence of the hSLNAC1 protein (SEQ ID NO : 1) can be labeled with <sup>32</sup>P using the Rediprime™ DNA labeling system (Amersham Life Science, Arlington, IL), according to manufacturer's instructions. After labeling, the probe can be purified using a CHROMA SPIN- 100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for hSLNAC1 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues can be obtained from Clontech and examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots can be mounted and exposed to film at -70°C overnight, and films developed according to standard procedures. It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims. The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are incorporated by reference.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5 (i) APPLICANT:  
    (A) NAME: Synthelabo  
    (B) STREET: 10 rue  
    (C) CITY: plessis  
    (E) COUNTRY: france  
10 (F) POSTAL CODE (ZIP): 92350  
  
    (ii) TITLE OF INVENTION: Sodium channel receptor  
  
    (iii) NUMBER OF SEQUENCES: 6  
15  
    (iv) COMPUTER READABLE FORM:  
        (A) MEDIUM TYPE: Floppy disk  
        (B) COMPUTER: IBM PC compatible  
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
20 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

25 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 1723 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: double  
    (D) TOPOLOGY: circular  
  
30 (ii) MOLECULE TYPE: cDNA to mRNA  
  
    (iii) HYPOTHETICAL: NO  
  
    (iv) ANTI-SENSE: NO  
35  
    (vi) ORIGINAL SOURCE:  
        (A) ORGANISM: Homo sapiens  
        (F) TISSUE TYPE: Cerebellum  
  
40 (vii) IMMEDIATE SOURCE:  
    (B) CLONE: SLNAC1  
  
    (ix) FEATURE:  
        (A) NAME/KEY: CDS  
45 (B) LOCATION: 13..1641  
        (D) OTHER INFORMATION: /codon\_start= 13  
                                /function= "sodium channel receptor"  
                                /product= "SLNAC1"  
                                /standard\_name= "slnac1"  
50                                /label= slnac1



## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION:1..12
- (D) OTHER INFORMATION:/product= "5' untranslated"

5

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION:1645..1723
- (D) OTHER INFORMATION:/product= "3' untranslated"

10

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION:1642..1644
- (D) OTHER INFORMATION:/product= "stop codon"

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCGGTTCTGG CC ATG AAG CCC ACC TCA GGC CCA GAG GAG GCC CGG CGG  
48

20

Met Lys Pro Thr Ser Gly Pro Glu Glu Ala Arg Arg  
1 5 10

CCA GCC TCG GAC ATC CGC GTG TTC GCC AGC AAC TGC TCG ATG CAC GGG  
96

25

Pro Ala Ser Asp Ile Arg Val Phe Ala Ser Asn Cys Ser Met His Gly  
15 20 25

CTG GGC CAC GTC TTC GGG CCA GGC AGC CTG AGC CTG CGC CGG GGG ATG  
144

30

Leu Gly His Val Phe Gly Pro Gly Ser Leu Ser Leu Arg Arg Gly Met  
30 35 40

TGG GCA GCG GCC GTG GTC CTG TCA GTG GCC ACC TTC CTC TAC CAG GTG  
192

35

Trp Ala Ala Ala Val Val Leu Ser Val Ala Thr Phe Leu Tyr Gln Val  
45 50 55 60

GCT GAG AGG GTG CGC TAC TAC AGG GAG TTC CAC CAC CAG ACT GCC CTG  
240

40

Ala Glu Arg Val Arg Tyr Tyr Arg Glu Phe His His Gln Thr Ala Leu  
65 70 75

GAT GAG CGA GAA AGC CAC CGG CTC ATC TTC CCG GCT GTC ACC CTG TGC  
288

45

Asp Glu Arg Glu Ser His Arg Leu Ile Phe Pro Ala Val Thr Leu Cys  
80 85 90

AAC ATC AAC CCA CTG CGC CGC TCG CGC CTA ACG CCC AAC GAC CTG CAC  
336

50

Asn Ile Asn Pro Leu Arg Arg Ser Arg Leu Thr Pro Asn Asp Leu His

	95	100	105
	TGG GCT GGG TCT GCG CTG CTG GGC CTG GAT CCC GCA GAG CAC GCC GCC		
	384		
5	Trp Ala Gly Ser Ala Leu Leu Gly Leu Asp Pro Ala Glu His Ala Ala		
	110	115	120
	TTC CTG CGC GCC CTG GGC CGG CCC CCT GCA CCG CCC GGC TTC ATG CCC		
	432		
10	Phe Leu Arg Ala Leu Gly Arg Pro Pro Ala Pro Pro Gly Phe Met Pro		
	125	130	135 140
	AGT CCC ACC TTT GAC ATG GCG CAA CTC TAT GCC CGT GCT GGG CAC TCC		
	480		
15	Ser Pro Thr Phe Asp Met Ala Gln Leu Tyr Ala Arg Ala Gly His Ser		
	145	150	155
	CTG GAT GAC ATG CTG CTG GAC TGT CGC TTC CGT GGC CAA CCT TGT GGG		
	528		
20	Leu Asp Asp Met Leu Leu Asp Cys Arg Phe Arg Gly Gln Pro Cys Gly		
	160	165	170
	CCT GAG AAC TTC ACC ACG ATC TTC ACC CGG ATG GGA AAG TGC TAC ACA		
	576		
25	Pro Glu Asn Phe Thr Thr Ile Phe Thr Arg Met Gly Lys Cys Tyr Thr		
	175	180	185
	TTT AAC TCT GGC GCT GAT GGG GCA GAG CTG CTC ACC ACT ACT AGG GGT		
	624		
30	Phe Asn Ser Gly Ala Asp Gly Ala Glu Leu Leu Thr Thr Thr Arg Gly		
	190	195	200
	GGC ATG GGC AAT GGG CTG GAC ATC ATG CTG GAC GTG CAG CAG GAG GAA		
	672		
35	Gly Met Gly Asn Gly Leu Asp Ile Met Leu Asp Val Gln Gln Glu Glu		
	205	210	215 220
	TAT CTA CCT GTG TGG AGG GAC AAT GAG GAG ACC CCG TTT GAG GTG GGG		
	720		
40	Tyr Leu Pro Val Trp Arg Asp Asn Glu Glu Thr Pro Phe Glu Val Gly		
	225	230	235
	ATC CGA GTG CAG ATC CAC AGC CAG GAG GAG CCG CCC ATC ATC GAT CAG		
	768		
45	Ile Arg Val Gln Ile His Ser Gln Glu Glu Pro Pro Ile Ile Asp Gln		
	240	245	250
	CTG GGC TTG GGG GTG TCC CCG GGC TAC CAG ACC TTT GTT TCT TGC CAG		
	816		
50	Leu Gly Leu Gly Val Ser Pro Gly Tyr Gln Thr Phe Val Ser Cys Gln		
	255	260	265

CAG CAG CAG CTG AGC TTC CTG CCA CCG CCC TGG GGC GAT TGC AGT TCA  
 864  
 Gln Gln Gln Leu Ser Phe Leu Pro Pro Pro Trp Gly Asp Cys Ser Ser  
 270 275 280

5 GCA TCT CTG AAC CCC AAC TAT GAG CCA GAG CCC TCT GAT CCC CTA GGC  
 912  
 Ala Ser Leu Asn Pro Asn Tyr Glu Pro Glu Pro Ser Asp Pro Leu Gly  
 285 290 295 300

10 TCC CCC AGC CCC AGC CCC AGC CCT CCC TAT ACC CTT ATG GGG TGT CGC  
 960  
 Ser Pro Ser Pro Ser Pro Ser Pro Pro Tyr Thr Leu Met Gly Cys Arg  
 305 310 315

15 CTG GCC TGC GAA ACC CGC TAC GTG GCT CGG AAG TGC GGC TGC CGA ATG  
 1008  
 Leu Ala Cys Glu Thr Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met  
 320 325 330

20 GTG TAC ATG CCA GGC GAC GTG CCA GTG TGC AGC CCC CAG CAG TAC AAG  
 1056  
 Val Tyr Met Pro Gly Asp Val Pro Val Cys Ser Pro Gln Gln Tyr Lys  
 335 340 345

25 AAC TGT GCC CAC CCG GCC ATA GAT GCC ATG CTT CGC AAG GAC TCG TGC  
 1104  
 Asn Cys Ala His Pro Ala Ile Asp Ala Met Leu Arg Lys Asp Ser Cys  
 350 355 360

30 GCC TGC CCC AAC CCG TGC GCC AGC ACG CGC TAC GCC AAG GAG CTC TCC  
 1152  
 Ala Cys Pro Asn Pro Cys Ala Ser Thr Arg Tyr Ala Lys Glu Leu Ser  
 365 370 375 380

35 ATG GTG CGG ATC CCG AGC CGC GCC GCC GCG CGC TTC CTG GCC CGG AAG  
 1200  
 Met Val Arg Ile Pro Ser Arg Ala Ala Ala Arg Phe Leu Ala Arg Lys  
 385 390 395

40 CTC AAC CGC AGC GAG GCC TAC ATC GCG GAG AAC GTG CTG GCC CTG GAC  
 1248  
 Leu Asn Arg Ser Glu Ala Tyr Ile Ala Glu Asn Val Leu Ala Leu Asp  
 400 405 410

45 ATC TTC TTT GAG GCC CTC AAC TAT GAG ACC GTG GAG CAG AAG AAG GCC  
 1296  
 Ile Phe Phe Glu Ala Leu Asn Tyr Glu Thr Val Glu Gln Lys Lys Ala  
 415 420 425

50 TAT GAG ATG TCA GAG CTG CTT GGT GAC ATT GGG GGC CAG ATG GGG CTG  
 1344

Tyr Glu Met Ser Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu  
 430 435 440  
 TTC ATC GGG GCC AGC CTG CTC ACC ATC CTC GAG ATC CTA GAC TAC CTC  
 1392  
 5 Phe Ile Gly Ala Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu  
 445 450 455 460  
 TGT GAG GTG TTC CGA GAC AAG GTC CTG GGA TAT TTC TGG AAC CGA CAG  
 1440  
 10 Cys Glu Val Phe Arg Asp Lys Val Leu Gly Tyr Phe Trp Asn Arg Gln  
 465 470 475  
 CAC TCC CAA AGG CAC TCC AGC ACC AAT CTG ACC TCC CAC CCC TCC CTG  
 1488  
 15 His Ser Gln Arg His Ser Ser Thr Asn Leu Thr Ser His Pro Ser Leu  
 480 485 490  
 TGC CGT CAC CAA GAC TCT CTC CGC CTC CCA CCG CAC CTG CTA CCT TGT  
 1536  
 20 Cys Arg His Gln Asp Ser Leu Arg Leu Pro Pro His Leu Leu Pro Cys  
 495 500 505  
 CAC ACA GCT CTA GAC CTG CTG TCT GTG TCC TCG GAG CCC CGC CCT GAC  
 1584  
 25 His Thr Ala Leu Asp Leu Leu Ser Val Ser Ser Glu Pro Arg Pro Asp  
 510 515 520  
 ATC CTG GAC ATG CCT AGC CTG CAC GTA GCT TTT CCG TCT TCA CCC CAA  
 1632  
 30 Ile Leu Asp Met Pro Ser Leu His Val Ala Phe Pro Ser Ser Pro Gln  
 525 530 535 540  
 ATA AAG TCC TAATGCATCA AAAAAAAAAA AAAAAAAAAA CTCGAGGGGG  
 1681  
 35 Ile Lys Ser

GGCCCGGTAC CCAATTCGCC CTATAGTGAG TCGTATTACA AT  
 1723

40

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 543 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

35

	Met	Lys	Pro	Thr	Ser	Gly	Pro	Glu	Glu	Ala	Arg	Arg	Pro	Ala	Ser	Asp	
	1				5					10					15		
	Ile	Arg	Val	Phe	Ala	Ser	Asn	Cys	Ser	Met	His	Gly	Leu	Gly	His	Val	
				20					25					30			
5																	
	Phe	Gly	Pro	Gly	Ser	Leu	Ser	Leu	Arg	Arg	Gly	Met	Trp	Ala	Ala	Ala	
				35				40					45				
	Val	Val	Leu	Ser	Val	Ala	Thr	Phe	Leu	Tyr	Gln	Val	Ala	Glu	Arg	Val	
10			50				55					60					
	Arg	Tyr	Tyr	Arg	Glu	Phe	His	His	Gln	Thr	Ala	Leu	Asp	Glu	Arg	Glu	
	65					70					75					80	
	Ser	His	Arg	Leu	Ile	Phe	Pro	Ala	Val	Thr	Leu	Cys	Asn	Ile	Asn	Pro	
15					85					90					95		
	Leu	Arg	Arg	Ser	Arg	Leu	Thr	Pro	Asn	Asp	Leu	His	Trp	Ala	Gly	Ser	
				100					105					110			
20																	
	Ala	Leu	Leu	Gly	Leu	Asp	Pro	Ala	Glu	His	Ala	Ala	Phe	Leu	Arg	Ala	
				115				120					125				
	Leu	Gly	Arg	Pro	Pro	Ala	Pro	Pro	Gly	Phe	Met	Pro	Ser	Pro	Thr	Phe	
25				130			135					140					
	Asp	Met	Ala	Gln	Leu	Tyr	Ala	Arg	Ala	Gly	His	Ser	Leu	Asp	Asp	Met	
	145				150					155						160	
	Leu	Leu	Asp	Cys	Arg	Phe	Arg	Gly	Gln	Pro	Cys	Gly	Pro	Glu	Asn	Phe	
30					165				170						175		
	Thr	Thr	Ile	Phe	Thr	Arg	Met	Gly	Lys	Cys	Tyr	Thr	Phe	Asn	Ser	Gly	
				180					185					190			
35																	
	Ala	Asp	Gly	Ala	Glu	Leu	Leu	Thr	Thr	Thr	Arg	Gly	Gly	Met	Gly	Asn	
				195				200					205				
	Gly	Leu	Asp	Ile	Met	Leu	Asp	Val	Gln	Gln	Glu	Glu	Tyr	Leu	Pro	Val	
40				210			215					220					
	Trp	Arg	Asp	Asn	Glu	Glu	Thr	Pro	Phe	Glu	Val	Gly	Ile	Arg	Val	Gln	
	225				230						235					240	
	Ile	His	Ser	Gln	Glu	Glu	Pro	Pro	Ile	Ile	Asp	Gln	Leu	Gly	Leu	Gly	
45					245				250						255		
	Val	Ser	Pro	Gly	Tyr	Gln	Thr	Phe	Val	Ser	Cys	Gln	Gln	Gln	Gln	Leu	
				260				265						270			
50																	
	Ser	Phe	Leu	Pro	Pro	Pro	Trp	Gly	Asp	Cys	Ser	Ser	Ala	Ser	Leu	Asn	

	275		280		285
	Pro Asn Tyr Glu Pro Glu	Pro Ser Asp Pro Leu Gly Ser Pro Ser Pro			
	290	295	300		
5	Ser Pro Ser Pro Pro Tyr Thr Leu Met Gly Cys Arg Leu Ala Cys Glu				
	305	310	315	320	
	Thr Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met Val Tyr Met Pro				
		325	330	335	
10	Gly Asp Val Pro Val Cys Ser Pro Gln Gln Tyr Lys Asn Cys Ala His				
		340	345	350	
	Pro Ala Ile Asp Ala Met Leu Arg Lys Asp Ser Cys Ala Cys Pro Asn				
15		355	360	365	
	Pro Cys Ala Ser Thr Arg Tyr Ala Lys Glu Leu Ser Met Val Arg Ile				
		370	375	380	
20	Pro Ser Arg Ala Ala Ala Arg Phe Leu Ala Arg Lys Leu Asn Arg Ser				
		385	390	395	400
	Glu Ala Tyr Ile Ala Glu Asn Val Leu Ala Leu Asp Ile Phe Phe Glu				
		405	410	415	
25	Ala Leu Asn Tyr Glu Thr Val Glu Gln Lys Lys Ala Tyr Glu Met Ser				
		420	425	430	
	Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala				
30		435	440	445	
	Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu Cys Glu Val Phe				
		450	455	460	
35	Arg Asp Lys Val Leu Gly Tyr Phe Trp Asn Arg Gln His Ser Gln Arg				
		465	470	475	480
	His Ser Ser Thr Asn Leu Thr Ser His Pro Ser Leu Cys Arg His Gln				
		485	490	495	
40	Asp Ser Leu Arg Leu Pro Pro His Leu Leu Pro Cys His Thr Ala Leu				
		500	505	510	
	Asp Leu Leu Ser Val Ser Ser Glu Pro Arg Pro Asp Ile Leu Asp Met				
45		515	520	525	
	Pro Ser Leu His Val Ala Phe Pro Ser Ser Pro Gln Ile Lys Ser				
		530	535	540	

## (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 1711 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: circular

10 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens  
 (F) TISSUE TYPE: Cerebellum

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

CTGGAGCTCC ACCGCGGTGG CGGCCGCTCT AGAACTAGTG GATCCCCCGG GCTGCAGGAA  
 25 60

TTCGGCACGA GCTCG ATG CAC GGG CTG GGC CAC GTC TTC GGG CCA GGC AGC  
 111

Met His Gly Leu Gly His Val Phe Gly Pro Gly Ser  
 1 5 10

30 CTG AGC CTG CGC CGG GGG ATG TGG GCA GCG GCC GTG GTC CTG TCA GTG  
 159

Leu Ser Leu Arg Arg Gly Met Trp Ala Ala Ala Val Val Leu Ser Val  
 15 20 25

35 GCC ACC TTC CTC TAC CAG GTG GCT GAG AGG GTG CGC TAC TAC AGG GAG  
 207

Ala Thr Phe Leu Tyr Gln Val Ala Glu Arg Val Arg Tyr Tyr Arg Glu  
 40 30 35 40

TTC CAC CAC CAG ACT GCC CTG GAT GAG CGA GAA AGC CAC CGG CTC ATC  
 255

Phe His His Gln Thr Ala Leu Asp Glu Arg Glu Ser His Arg Leu Ile  
 45 45 50 55 60

TTC CCG GCT GTC ACC CTG TGC AAC ATC AAC CCA CTG CGC CGC TCG CGC  
 303

Phe Pro Ala Val Thr Leu Cys Asn Ile Asn Pro Leu Arg Arg Ser Arg  
 50 65 70 75

CTA ACG CCC AAC GAC CTG CAC TGG GCT GGG TCT GCG CTG CTG GGC CTG  
 351  
 Leu Thr Pro Asn Asp Leu His Trp Ala Gly Ser Ala Leu Leu Gly Leu  
                     80                    85                    90

5 GAT CCC GCA GAG CAC GCC GCC TTC CTG CGC GCC CTG GGC CGG CCC CCT  
 399  
 Asp Pro Ala Glu His Ala Ala Phe Leu Arg Ala Leu Gly Arg Pro Pro  
                     95                    100                    105

10 GCA CCG CCC GGC TTC ATG CCC AGT CCC ACC TTT GAC ATG GCG CAA CTC  
 447  
 Ala Pro Pro Gly Phe Met Pro Ser Pro Thr Phe Asp Met Ala Gln Leu  
                     110                    115                    120

15 TAT GCC CGT GCT GGG CAC TCC CTG GAT GAC ATG CTG CTG GAC TGT CGC  
 495  
 Tyr Ala Arg Ala Gly His Ser Leu Asp Asp Met Leu Leu Asp Cys Arg  
 125                    130                    135                    140

20 TTC CGT GGC CAA CCT TGT GGG CCT GAG AAC TTC ACC ACG ATC TTC ACC  
 543  
 Phe Arg Gly Gln Pro Cys Gly Pro Glu Asn Phe Thr Thr Ile Phe Thr  
                     145                    150                    155

25 CGG ATG GGA AAG TGC TAC ACA TTT AAC TCT GGC GCT GAT GGG GCA GAG  
 591  
 Arg Met Gly Lys Cys Tyr Thr Phe Asn Ser Gly Ala Asp Gly Ala Glu  
                     160                    165                    170

30 CTG CTC ACC ACT ACT AGG GGT GGC ATG GGC AAT GGG CTG GAC ATC ATG  
 639  
 Leu Leu Thr Thr Thr Arg Gly Gly Met Gly Asn Gly Leu Asp Ile Met  
                     175                    180                    185

35 CTG GAC GTG CAG CAG GAG GAA TAT CTA CCT GTG TGG AGG GAC AAT GAG  
 687  
 Leu Asp Val Gln Gln Glu Glu Tyr Leu Pro Val Trp Arg Asp Asn Glu  
                     190                    195                    200

40 GAG ACC CCG TTT GAG GTG GGG ATC CGA GTG CAG ATC CAC AGC CAG GAG  
 735  
 Glu Thr Pro Phe Glu Val Gly Ile Arg Val Gln Ile His Ser Gln Glu  
 205                    210                    215                    220

45 GAG CCG CCC ATC ATC GAT CAG CTG GGC TTG GGG GTG TCC CCG GGC TAC  
 783  
 Glu Pro Pro Ile Ile Asp Gln Leu Gly Leu Gly Val Ser Pro Gly Tyr  
                     225                    230                    235

50 CAG ACC TTT GTT TCT TGC CAG CAG CAG CAG CTG AGC TTC CTG CCA CCG  
 831



Gln Thr Phe Val Ser Cys Gln Gln Gln Gln Leu Ser Phe Leu Pro Pro  
 240 245 250  
 CCC TGG GGC GAT TGC AGT TCA GCA TCT CTG AAC CCC AAC TAT GAG CCA  
 879  
 5 Pro Trp Gly Asp Cys Ser Ser Ala Ser Leu Asn Pro Asn Tyr Glu Pro  
 255 260 265  
 GAG CCC TCT GAT CCC CTA GGC TCC CCC AGC CCC AGC CCC AGC CCT CCC  
 927  
 10 Glu Pro Ser Asp Pro Leu Gly Ser Pro Ser Pro Ser Pro Ser Pro Pro  
 270 275 280  
 TAT ACC CTT ATG GGG TGT CGC CTG GCC TGC GAA ACC CGC TAC GTG GCT  
 975  
 15 Tyr Thr Leu Met Gly Cys Arg Leu Ala Cys Glu Thr Arg Tyr Val Ala  
 285 290 295 300  
 CGG AAG TGC GGC TGC CGA ATG GTG TAC ATG CCA GGC GAC GTG CCA GTG  
 1023  
 20 Arg Lys Cys Gly Cys Arg Met Val Tyr Met Pro Gly Asp Val Pro Val  
 305 310 315  
 TGC AGC CCC CAG CAG TAC AAG AAC TGT GCC CAC CCG GCC ATA GAT GCC  
 1071  
 25 Cys Ser Pro Gln Gln Tyr Lys Asn Cys Ala His Pro Ala Ile Asp Ala  
 320 325 330  
 ATG CTT CGC AAG GAC TCG TGC GCC TGC CCC AAC CCG TGC GCC AGC ACG  
 1119  
 30 Met Leu Arg Lys Asp Ser Cys Ala Cys Pro Asn Pro Cys Ala Ser Thr  
 335 340 345  
 CGC TAC GCC AAG GAG CTC TCC ATG GTG CGG ATC CCG AGC CGC GCC GCC  
 1167  
 35 Arg Tyr Ala Lys Glu Leu Ser Met Val Arg Ile Pro Ser Arg Ala Ala  
 350 355 360  
 GCG CGC TTC CTG GCC CGG AAG CTC AAC CGC AGC GAG GCC TAC ATC GCG  
 1215  
 40 Ala Arg Phe Leu Ala Arg Lys Leu Asn Arg Ser Glu Ala Tyr Ile Ala  
 365 370 375 380  
 GAG AAC GTG CTG GCC CTG GAC ATC TTC TTT GAG GCC CTC AAC TAT GAG  
 1263  
 45 Glu Asn Val Leu Ala Leu Asp Ile Phe Phe Glu Ala Leu Asn Tyr Glu  
 385 390 395  
 ACC GTG GAG CAG AAG AAG GCC TAT GAG ATG TCA GAG CTG CTT GGT GAC  
 1311  
 50 Thr Val Glu Gln Lys Lys Ala Tyr Glu Met Ser Glu Leu Leu Gly Asp

40

400 405 410  
 ATT GGG GGC CAG ATG GGG CTG TTC ATC GGG GCC AGC CTG CTC ACC ATC  
 1359  
 Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Leu Leu Thr Ile  
 5 415 420 425  
 CTC GAG ATC CTA GAC TAC CTC TGT GAG GTG TTC CGA GAC AAG GTC CTG  
 1407  
 Leu Glu Ile Leu Asp Tyr Leu Cys Glu Val Phe Arg Asp Lys Val Leu  
 10 430 435 440  
 GGA TAT TTC TGG AAC CGA CAG CAC TCC CAA AGG CAC TCC AGC ACC AAT  
 1455  
 Gly Tyr Phe Trp Asn Arg Gln His Ser Gln Arg His Ser Ser Thr Asn  
 15 445 450 455 460  
 CTG ACC TCC CAC CCC TCC CTG TGC CGT CAC CAA GAC TCT CTC CGC CTC  
 1503  
 Leu Thr Ser His Pro Ser Leu Cys Arg His Gln Asp Ser Leu Arg Leu  
 20 465 470 475  
 CCA CCG CAC CTG CTA CCT TGT CAC ACA GCT CTA GAC CTG CTG TCT GTG  
 1551  
 Pro Pro His Leu Leu Pro Cys His Thr Ala Leu Asp Leu Leu Ser Val  
 25 480 485 490  
 TCC TCG GAG CCC CGC CCT GAC ATC CTG GAC ATG CCT AGC CTG CAC GTA  
 1599  
 Ser Ser Glu Pro Arg Pro Asp Ile Leu Asp Met Pro Ser Leu His Val  
 30 495 500 505  
 GCT TTT CCG TCT TCA CCC CAA ATA AAG TCC TAA TGCATCAAAA AAAAAAAAAA  
 1652  
 Ala Phe Pro Ser Ser Pro Gln Ile Lys Ser \*  
 35 510 515  
 AAAAAAACTC GAGGGGGGGC CCGGTACCCA ATTCGCCCTA TAGTGAGTCG TATTACAAT  
 1711

40

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

45

(A) LENGTH: 519 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

50

41

Met His Gly Leu Gly His Val Phe Gly Pro Gly Ser Leu Ser Leu Arg  
 1 5 10 15  
 Arg Gly Met Trp Ala Ala Ala Val Val Leu Ser Val Ala Thr Phe Leu  
 20 25 30  
 5 Tyr Gln Val Ala Glu Arg Val Arg Tyr Tyr Arg Glu Phe His His Gln  
 35 40 45  
 10 Thr Ala Leu Asp Glu Arg Glu Ser His Arg Leu Ile Phe Pro Ala Val  
 50 55 60  
 Thr Leu Cys Asn Ile Asn Pro Leu Arg Arg Ser Arg Leu Thr Pro Asn  
 65 70 75 80  
 15 Asp Leu His Trp Ala Gly Ser Ala Leu Leu Gly Leu Asp Pro Ala Glu  
 85 90 95  
 His Ala Ala Phe Leu Arg Ala Leu Gly Arg Pro Pro Ala Pro Pro Gly  
 100 105 110  
 20 Phe Met Pro Ser Pro Thr Phe Asp Met Ala Gln Leu Tyr Ala Arg Ala  
 115 120 125  
 Gly His Ser Leu Asp Asp Met Leu Leu Asp Cys Arg Phe Arg Gly Gln  
 130 135 140  
 25 Pro Cys Gly Pro Glu Asn Phe Thr Thr Ile Phe Thr Arg Met Gly Lys  
 145 150 155 160  
 30 Cys Tyr Thr Phe Asn Ser Gly Ala Asp Gly Ala Glu Leu Leu Thr Thr  
 165 170 175  
 Thr Arg Gly Gly Met Gly Asn Gly Leu Asp Ile Met Leu Asp Val Gln  
 180 185 190  
 35 Gln Glu Glu Tyr Leu Pro Val Trp Arg Asp Asn Glu Glu Thr Pro Phe  
 195 200 205  
 Glu Val Gly Ile Arg Val Gln Ile His Ser Gln Glu Glu Pro Pro Ile  
 210 215 220  
 40 Ile Asp Gln Leu Gly Leu Gly Val Ser Pro Gly Tyr Gln Thr Phe Val  
 225 230 235 240  
 45 Ser Cys Gln Gln Gln Gln Leu Ser Phe Leu Pro Pro Pro Trp Gly Asp  
 245 250 255  
 Cys Ser Ser Ala Ser Leu Asn Pro Asn Tyr Glu Pro Glu Pro Ser Asp  
 260 265 270  
 50

42

Pro Leu Gly Ser Pro Ser Pro Ser Pro Ser Pro Pro Tyr Thr Leu Met  
 275 280 285  
 Gly Cys Arg Leu Ala Cys Glu Thr Arg Tyr Val Ala Arg Lys Cys Gly  
 290 295 300  
 5  
 Cys Arg Met Val Tyr Met Pro Gly Asp Val Pro Val Cys Ser Pro Gln  
 305 310 315 320  
 10 Gln Tyr Lys Asn Cys Ala His Pro Ala Ile Asp Ala Met Leu Arg Lys  
 325 330 335  
 Asp Ser Cys Ala Cys Pro Asn Pro Cys Ala Ser Thr Arg Tyr Ala Lys  
 340 345 350  
 15 Glu Leu Ser Met Val Arg Ile Pro Ser Arg Ala Ala Ala Arg Phe Leu  
 355 360 365  
 Ala Arg Lys Leu Asn Arg Ser Glu Ala Tyr Ile Ala Glu Asn Val Leu  
 20 370 375 380  
 Ala Leu Asp Ile Phe Phe Glu Ala Leu Asn Tyr Glu Thr Val Glu Gln  
 385 390 395 400  
 25 Lys Lys Ala Tyr Glu Met Ser Glu Leu Leu Gly Asp Ile Gly Gly Gln  
 405 410 415  
 Met Gly Leu Phe Ile Gly Ala Ser Leu Leu Thr Ile Leu Glu Ile Leu  
 30 420 425 430  
 Asp Tyr Leu Cys Glu Val Phe Arg Asp Lys Val Leu Gly Tyr Phe Trp  
 435 440 445  
 Asn Arg Gln His Ser Gln Arg His Ser Ser Thr Asn Leu Thr Ser His  
 35 450 455 460  
 Pro Ser Leu Cys Arg His Gln Asp Ser Leu Arg Leu Pro Pro His Leu  
 465 470 475 480  
 40 Leu Pro Cys His Thr Ala Leu Asp Leu Leu Ser Val Ser Ser Glu Pro  
 485 490 495  
 Arg Pro Asp Ile Leu Asp Met Pro Ser Leu His Val Ala Phe Pro Ser  
 500 505 510  
 45 Ser Pro Gln Ile Lys Ser \*  
 515

50 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1650 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: circular

5 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens  
 (F) TISSUE TYPE: Cerebellum

15 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1647  
 (D) OTHER INFORMATION: /codon\_start= 1  
 /function= "sodium channel receptor"  
 /product= "SLNAC1"  
 /standard\_name= "slnac1"  
 /label= slnac1

20

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATG AAG CCC ACC TCA GGC CCA GAG GAG GCC CGG CGG CCA GCC TCG GAC  
 48

30 Met Lys Pro Thr Ser Gly Pro Glu Glu Ala Arg Arg Pro Ala Ser Asp  
 1 5 10 15

ATC CGC GTG TTC GCC AGC AAC TGC TCG ATG CAC GGG CTG GGC CAC GTC  
 96

35 Ile Arg Val Phe Ala Ser Asn Cys Ser Met His Gly Leu Gly His Val  
 20 25 30

TTC GGG CCA GGC AGC CTG AGC CTG CGC CGG GGG ATG TGG GCA GCG GCC  
 144

40 Phe Gly Pro Gly Ser Leu Ser Leu Arg Arg Gly Met Trp Ala Ala Ala  
 35 40 45

GTG GTC CTG TCA GTG GCC ACC TTC CTC TAC CAG GTG GCT GAG AGG GTG  
 192

45 Val Val Leu Ser Val Ala Thr Phe Leu Tyr Gln Val Ala Glu Arg Val  
 50 55 60

CGC TAC TAC AGG GAG TTC CAC CAC CAG ACT GCC CTG GAT GAG CGA GAA  
 240

50 Arg Tyr Tyr Arg Glu Phe His His Gln Thr Ala Leu Asp Glu Arg Glu  
 65 70 75 80

AGC CAC CGG CTC ATC TTC CCG GCT GTC ACC CTG TGC AAC ATC AAC CCA  
 288  
 Ser His Arg Leu Ile Phe Pro Ala Val Thr Leu Cys Asn Ile Asn Pro  
                     85                                    90                                    95

5 CTG CGC CGC TCG CGC CTA ACG CCC AAC GAC CTG CAC TGG GCT GGG TCT  
 336  
 Leu Arg Arg Ser Arg Leu Thr Pro Asn Asp Leu His Trp Ala Gly Ser  
                     100                                    105                                    110

10 GCG CTG CTG GGC CTG GAT CCC GCA GAG CAC GCC GCC TTC CTG CGC GCC  
 384  
 Ala Leu Leu Gly Leu Asp Pro Ala Glu His Ala Ala Phe Leu Arg Ala  
                     115                                    120                                    125

15 CTG GGC CGG CCC CCT GCA CCG CCC GGC TTC ATG CCC AGT CCC ACC TTT  
 432  
 Leu Gly Arg Pro Pro Ala Pro Pro Gly Phe Met Pro Ser Pro Thr Phe  
                     130                                    135                                    140

20 GAC ATG GCG CAA CTC TAT GCC CGT GCT GGG CAC TCC CTG GAT GAC ATG  
 480  
 Asp Met Ala Gln Leu Tyr Ala Arg Ala Gly His Ser Leu Asp Asp Met  
                     145                                    150                                    155                                    160

25 CTG CTG GAC TGT CGC TTC CGT GGC CAA CCT TGT GGG CCT GAG AAC TTC  
 528  
 Leu Leu Asp Cys Arg Phe Arg Gly Gln Pro Cys Gly Pro Glu Asn Phe  
                     165                                    170                                    175

30 ACC ACG ATC TTC ACC CGG ATG GGA AAG TGC TAC ACA TTT AAC TCT GGC  
 576  
 Thr Thr Ile Phe Thr Arg Met Gly Lys Cys Tyr Thr Phe Asn Ser Gly  
                     180                                    185                                    190

35 GCT GAT GGG GCA GAG CTG CTC ACC ACT ACT AGG GGT GGC ATG GGC AAT  
 624  
 Ala Asp Gly Ala Glu Leu Leu Thr Thr Thr Arg Gly Gly Met Gly Asn  
                     195                                    200                                    205

40 GGG CTG GAC ATC ATG CTG GAC GTG CAG CAG GAG GAA TAT CTA CCT GTG  
 672  
 Gly Leu Asp Ile Met Leu Asp Val Gln Gln Glu Glu Tyr Leu Pro Val  
                     210                                    215                                    220

45 TGG AGG GAC AAT GAG GAG ACC CCG TTT GAG GTG GGG ATC CGA GTG CAG  
 720  
 Trp Arg Asp Asn Glu Glu Thr Pro Phe Glu Val Gly Ile Arg Val Gln  
                     225                                    230                                    235                                    240

50 ATC CAC AGC CAG GAG GAG CCG CCC ATC ATC GAT CAG CTG GGC TTG GGG  
 768

Ile His Ser Gln Glu Glu Pro Pro Ile Ile Asp Gln Leu Gly Leu Gly  
 245 250 255  
 GTG TCC CCG GGC TAC CAG ACC TTT GTT TCT TGC CAG CAG CAG CAG CTG  
 816  
 5 Val Ser Pro Gly Tyr Gln Thr Phe Val Ser Cys Gln Gln Gln Gln Leu  
 260 265 270  
 AGC TTC CTG CCA CCG CCC TGG GGC GAT TGC AGT TCA GCA TCT CTG AAC  
 864  
 10 Ser Phe Leu Pro Pro Pro Trp Gly Asp Cys Ser Ser Ala Ser Leu Asn  
 275 280 285  
 CCC AAC TAT GAG CCA GAG CCC TCT GAT CCC CTA GGC TCC CCC AGC CCC  
 912  
 15 Pro Asn Tyr Glu Pro Glu Pro Ser Asp Pro Leu Gly Ser Pro Ser Pro  
 290 295 300  
 AGC CCC AGC CCT CCC TAT ACC CTT ATG GGG TGT CGC CTG GCC TGC GAA  
 960  
 20 Ser Pro Ser Pro Pro Tyr Thr Leu Met Gly Cys Arg Leu Ala Cys Glu  
 305 310 315 320  
 ACC CGC TAC GTG GCT CGG AAG TGC GGC TGC CGA ATG GTG TAC ATG CCA  
 1008  
 25 Thr Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met Val Tyr Met Pro  
 325 330 335  
 GGC GAC GTG CCA GTG TGC AGC CCC CAG CAG TAC AAG AAC TGT GCC CAC  
 1056  
 Gly Asp Val Pro Val Cys Ser Pro Gln Gln Tyr Lys Asn Cys Ala His  
 340 345 350  
 35 CCG GCC ATA GAT GCC ATG CTT CGC AAG GAC TCG TGC GCC TGC CCC AAC  
 1104  
 Pro Ala Ile Asp Ala Met Leu Arg Lys Asp Ser Cys Ala Cys Pro Asn  
 355 360 365  
 40 CCG TGC GCC AGC ACG CGC TAC GCC AAG GAG CTC TCC ATG GTG CGG ATC  
 1152  
 Pro Cys Ala Ser Thr Arg Tyr Ala Lys Glu Leu Ser Met Val Arg Ile  
 370 375 380  
 45 CCG AGC CGC GCC GCC GCG CGC TTC CTG GCC CGG AAG CTC AAC CGC AGC  
 1200  
 Pro Ser Arg Ala Ala Ala Arg Phe Leu Ala Arg Lys Leu Asn Arg Ser  
 385 390 395 400  
 50 GAG GCC TAC ATC GCG GAG AAC GTG CTG GCC CTG GAC ATC TTC TTT GAG  
 1248

46

Glu Ala Tyr Ile Ala Glu Asn Val Leu Ala Leu Asp Ile Phe Phe Glu  
405 410 415

GCC CTC AAC TAT GAG ACC GTG GAG CAG AAG AAG GCC TAT GAG ATG TCA  
1296

5 Ala Leu Asn Tyr Glu Thr Val Glu Gln Lys Lys Ala Tyr Glu Met Ser  
420 425 430

GAG CTG CTT GGT GAC ATT GGG GGC CAG ATG GGG CTG TTC ATC GGG GCC  
1344

10 Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala  
435 440 445

AGC CTG CTC ACC ATC CTC GAG ATC CTA GAC TAC CTC TGT GAG GTG TTC  
1392

15 Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu Cys Glu Val Phe  
450 455 460

CGA GAC AAG GTC CTG GGA TAT TTC TGG AAC CGA CAG CAC TCC CAA AGG  
1440

20 Arg Asp Lys Val Leu Gly Tyr Phe Trp Asn Arg Gln His Ser Gln Arg  
465 470 475 480

CAC TCC AGC ACC AAT CTG CTT CAG GAA GGG CTG GGC AGC CAT CGA ACC  
1488

25 His Ser Ser Thr Asn Leu Leu Gln Glu Gly Leu Gly Ser His Arg Thr  
485 490 495

CAA GTT CCC CAC CTC AGC CTG GGC CCC AGC ACT CTG CTC TGT TCC GAA  
1536

30 Gln Val Pro His Leu Ser Leu Gly Pro Ser Thr Leu Leu Cys Ser Glu  
500 505 510

GAC CTC CCA CCC CTC CCT GTG CCG TCA CCA AGA CTC TCT CCG CCT CCC  
1584

35 Asp Leu Pro Pro Leu Pro Val Pro Ser Pro Arg Leu Ser Pro Pro Pro  
515 520 525

ACC GCA CCT GCT ACC TTG TCA CAC AGC TCT AGA CCT GCT GTC TGT GTC  
1632

40 Thr Ala Pro Ala Thr Leu Ser His Ser Ser Arg Pro Ala Val Cys Val  
530 535 540

CTC GGA GCC CCG CCC TGA  
1650

45 Leu Gly Ala Pro Pro  
545

50 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:



47

(A) LENGTH: 549 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```

Met Lys Pro Thr Ser Gly Pro Glu Glu Ala Arg Arg Pro Ala Ser Asp
 1             5             10             15

10 Ile Arg Val Phe Ala Ser Asn Cys Ser Met His Gly Leu Gly His Val
    20             25             30

Phe Gly Pro Gly Ser Leu Ser Leu Arg Arg Gly Met Trp Ala Ala Ala
    35             40             45
15 Val Val Leu Ser Val Ala Thr Phe Leu Tyr Gln Val Ala Glu Arg Val
    50             55             60

Arg Tyr Tyr Arg Glu Phe His His Gln Thr Ala Leu Asp Glu Arg Glu
20 65             70             75             80

Ser His Arg Leu Ile Phe Pro Ala Val Thr Leu Cys Asn Ile Asn Pro
    85             90             95

25 Leu Arg Arg Ser Arg Leu Thr Pro Asn Asp Leu His Trp Ala Gly Ser
    100             105             110

Ala Leu Leu Gly Leu Asp Pro Ala Glu His Ala Ala Phe Leu Arg Ala
    115             120             125
30 Leu Gly Arg Pro Pro Ala Pro Pro Gly Phe Met Pro Ser Pro Thr Phe
    130             135             140

Asp Met Ala Gln Leu Tyr Ala Arg Ala Gly His Ser Leu Asp Asp Met
35 145             150             155             160

Leu Leu Asp Cys Arg Phe Arg Gly Gln Pro Cys Gly Pro Glu Asn Phe
    165             170             175

40 Thr Thr Ile Phe Thr Arg Met Gly Lys Cys Tyr Thr Phe Asn Ser Gly
    180             185             190

Ala Asp Gly Ala Glu Leu Leu Thr Thr Thr Arg Gly Gly Met Gly Asn
    195             200             205
45 Gly Leu Asp Ile Met Leu Asp Val Gln Gln Glu Glu Tyr Leu Pro Val
    210             215             220

Trp Arg Asp Asn Glu Glu Thr Pro Phe Glu Val Gly Ile Arg Val Gln
50 225             230             235             240

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48

Ile His Ser Gln Glu Glu Pro Pro Ile Ile Asp Gln Leu Gly Leu Gly  
 245 250 255  
 Val Ser Pro Gly Tyr Gln Thr Phe Val Ser Cys Gln Gln Gln Gln Leu  
 260 265 270  
 5 Ser Phe Leu Pro Pro Pro Trp Gly Asp Cys Ser Ser Ala Ser Leu Asn  
 275 280 285  
 10 Pro Asn Tyr Glu Pro Glu Pro Ser Asp Pro Leu Gly Ser Pro Ser Pro  
 290 295 300  
 Ser Pro Ser Pro Pro Tyr Thr Leu Met Gly Cys Arg Leu Ala Cys Glu  
 305 310 315 320  
 15 Thr Arg Tyr Val Ala Arg Lys Cys Gly Cys Arg Met Val Tyr Met Pro  
 325 330 335  
 Gly Asp Val Pro Val Cys Ser Pro Gln Gln Tyr Lys Asn Cys Ala His  
 340 345 350  
 20 Pro Ala Ile Asp Ala Met Leu Arg Lys Asp Ser Cys Ala Cys Pro Asn  
 355 360 365  
 Pro Cys Ala Ser Thr Arg Tyr Ala Lys Glu Leu Ser Met Val Arg Ile  
 25 370 375 380  
 Pro Ser Arg Ala Ala Ala Arg Phe Leu Ala Arg Lys Leu Asn Arg Ser  
 385 390 395 400  
 30 Glu Ala Tyr Ile Ala Glu Asn Val Leu Ala Leu Asp Ile Phe Phe Glu  
 405 410 415  
 Ala Leu Asn Tyr Glu Thr Val Glu Gln Lys Lys Ala Tyr Glu Met Ser  
 420 425 430  
 35 Glu Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala  
 435 440 445  
 Ser Leu Leu Thr Ile Leu Glu Ile Leu Asp Tyr Leu Cys Glu Val Phe  
 40 450 455 460  
 Arg Asp Lys Val Leu Gly Tyr Phe Trp Asn Arg Gln His Ser Gln Arg  
 465 470 475 480  
 45 His Ser Ser Thr Asn Leu Leu Gln Glu Gly Leu Gly Ser His Arg Thr  
 485 490 495  
 Gln Val Pro His Leu Ser Leu Gly Pro Ser Thr Leu Leu Cys Ser Glu  
 500 505 510  
 50

Asp Leu Pro Pro Leu Pro Val Pro Ser Pro Arg Leu Ser Pro Pro Pro  
515 520 525

Thr Ala Pro Ala Thr Leu Ser His Ser Ser Arg Pro Ala Val Cys Val  
530 535 540

5

Leu Gly Ala Pro Pro  
545

**CLAIMS :**

1. An isolated polynucleotide selected from the group consisting of :
  - 5 (a) a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the hSLNAC1 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence,  
10 (b) a polynucleotide comprising a nucleotide sequence that has at least 80% identity to the cDNA insert deposited at the ATCC with Deposit Number 97987; or a nucleotide sequence complementary to said nucleotide sequence.  
15
2. A polynucleotide according to claim 1 which is DNA or RNA.
3. A polynucleotide according to one of claim 1 and 2  
20 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.
4. A polynucleotide according to claim 3 wherein said nucleotide sequence comprises the hSLNAC1 polypeptide  
25 encoding sequence contained in SEQ ID NO:1.
5. A polynucleotide according to claim 3 which is polynucleotide of SEQ ID NO: 1.
- 30 6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a hSLNAC1 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible  
35 host cell.
7. A host cell comprising the expression system of claim 6.
8. A process for producing a hSLNAC1 polypeptide comprising  
40 culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a hSLNAC1  
45 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a hSLNAC1 polypeptide.
- 50 10. A hSLNAC1 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence

of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

5 12. A hSLNAC1 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence encoded by the cDNA contained in ATCC 97987.

10 13. An antibody immunospecific for the hSLNAC1 polypeptide of one of claims 10 to 12.

14. Use of (a) a therapeutically effective amount of an agonist of hSLNAC1 polypeptide of claims 10 to 12, and/or (b) a polynucleotide according to one of claims 1 to 6 in a  
15 form so as to effect production of said hSLNAC1 polypeptide activity in vivo, for the manufacture of a medicament for the treatment of a subject in need of enhanced activity or expression of hSLNAC1 polypeptide.

20 15. Use of (a) a therapeutically effective amount of an antagonist of hSLNAC1 polypeptide of claims 10 to 12, and/or (b) a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said hSLNAC1 polypeptide and/or (c) a therapeutically effective amount of a  
25 polypeptide that competes with said hSLNAC1 polypeptide, for the manufacture of a medicament for the treatment of a subject having need to inhibit activity or expression of hSLNAC1 polypeptide.

30 16. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of hSLNAC1 polypeptide of one of claims 10 to 12 in a subject comprising:

35 (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said hSLNAC1 polypeptide in the genome of said subject; and/or  
(b) analyzing for the presence or amount of the hSLNAC1 polypeptide expression in a sample derived from said  
40 subject.

17. A process for identifying agonists to hSLNAC1 polypeptide of one of claims 10 to 12 comprising:

45 (a) contacting cells produced by claim 9 with a candidate compound; and

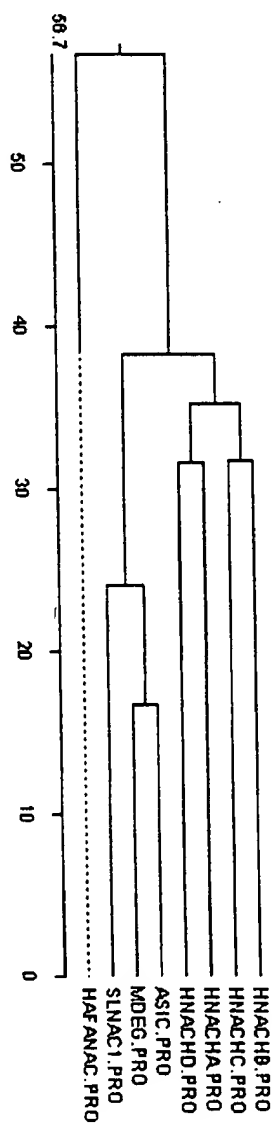
(b) determining whether the candidate compound effects a signal generated by activation of the hSLNAC1 polypeptide.  
50

18. An agonist identified by the method of claim 17.

19. A process for identifying antagonists to hSLNAC1 polypeptide of one of claims 10 to 12 comprising:
- (a) contacting said cell produced by claim 9 with an agonist; and
  - 5 (b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.
20. An antagonist identified by the method of claim 19.
- 10 21. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:4 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.
- 15 22. The polynucleotide sequence of SEQ ID NO:3.
23. A polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID
- 20 NO:4 over its entire length.
24. The polypeptide sequence of SEQ ID NO:4.
- 25 25. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:6 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.
- 30 26. The polynucleotide sequence of SEQ ID NO:5.
27. A polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID
- 35 NO:6 over its entire length.
28. The polypeptide sequence of SEQ ID NO:6.

1/5

Figure 1



[illegible]



3/5

Figure 2b, continued

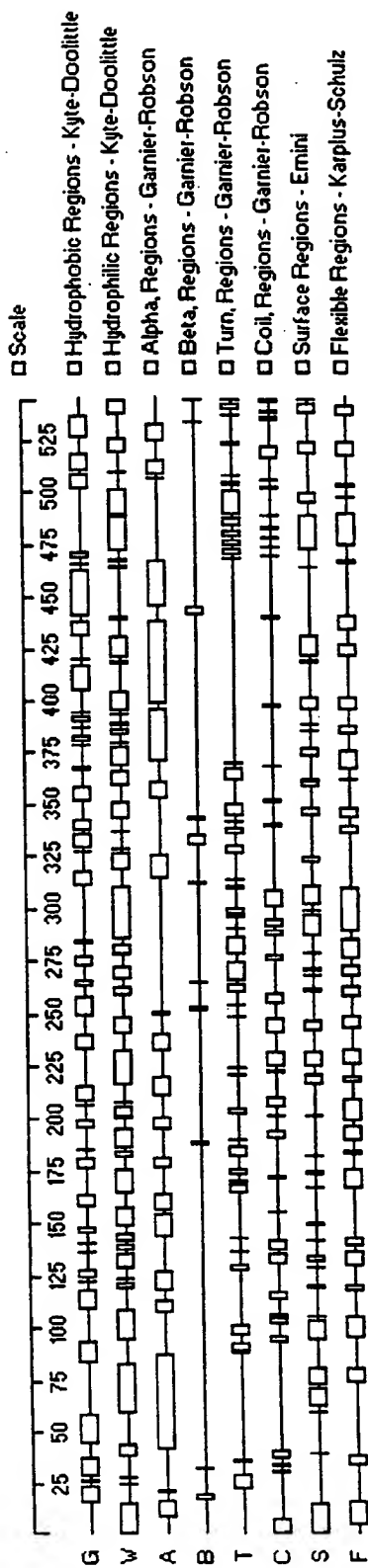
215	SLNACI
216	ASIC
217	MDEG
218	HNACHA
219	HNACHB
220	HNACHC
221	HNACHD
222	HAFANAC
223	
224	
225	
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Figure 2c, continued

463	... VFERDKVLGYFQNRQHSQRHSSTNLTSHPSLCKRHODSLRLPPHLLPCHTALDLLSVSSSEPRPDILDMPS	SLNACI
457	... VIKHRL... CRRGKCKQKEAKRSS... ADKGVALLSLDDVKREMPCESLRGHPAGMTYAANILP	ASIC
457	... LIKERL... LDLLGKEEEEGS... EDENMS... TCDMPNHEETISHTVNV.P	MDEG
580	MFLMLLRRFR.SRY.WSPGEGGRGAQEVASTLASSPPH.HFCPEPMSSLSSQPGPAPSPALTAPPPAYAT	HNACHA
550	ITIIKLVALAKSLRQRAQASYAGPPPTVAELVEAHTNFGFQPDIAFRSPNTGPYPSEQALPIPGTPPPPN	HNACHB
560	IIAREQWQKAKEWAWKQAPPCPEAPRSPQG... QDNPALEID.DDEFTFNSALHLPALGTQVPPTPPK	HNACHC
557	TLVLGGRRRLRRARQFSWPRASPASGASSIKFEASQMPPP... AGGTSDDDPEPSGPHPPRVVM	HNACHD
556	... VIELTGLVF... NSEKGLPRGPTTVNNNNGSNHESQSTSQHQLYNGYMDHDSHYSDSAGASV	HAFANAC
531	LHVAFPSSPQIKS	SLNACI
513	HHPARGTFEDFT... C	ASIC
500	LQTALGLLEELAC	MDEG
647	LGRPSPPGGSAGASSSTCPLG... GP	HNACHA
620	YDSLRLQPL... DVIESDSEGDAL	HNACHB
627	YNTLRLERAFSNQLTDTQMLDEL	HNACHC
614	LPGVLAGVSAEEWAGPQPLETLDI	HNACHD
615	FDPRRGVESPV	HAFANAC

**Figure 3**



# INTERNATIONAL SEARCH REPORT

Int. onal Application No

PCT/EP 98/02884

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/18 C12N5/10 A61K38/17  
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMEST8 DATABASE Accession number AA428361 Hillier L. et al. 25-MAY-1997 (Rel. 52, Created) similar to TR:G1256017 G1256017 SODIUM CHANNEL 1 XP002080468 see the whole document ---	1-6, 21-28
Y	GARCIA-ANOVEROS J ET AL: "BNaC1 and BNaC2 constitute a new family of human neuronal sodium channels related to degenerins and epithelial sodium channels." PROC NATL ACAD SCI U S A, FEB 18 1997, 94 (4) P1459-64, XP002051359 UNITED STATES see abstract; figure 2 ---	1-13, 16, 17, 21-28
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

13 October 1998

Date of mailing of the international search report

27/10/1998

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Gurdjian, D

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 98/02884

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 01577 A (UNIV LONDON) 16 January 1997 see claims 1-20 ---	16, 17, 21-28
Y	COREY DP ET AL: "Mechanosensation and the DEG/ENaC ion channels 'comment!'" SCIENCE, JUL 19 1996, 273 (5273) P323-4, XP002051360 UNITED STATES see page 323, right-hand column, paragraph 2; figure 1 ---	1-13, 21-28
A	WALDMANN R ET AL: "The mammalian degenerin MDEG, an amiloride-sensitive cation channel activated by mutations causing neurodegeneration in Caenorhabditis elegans." J BIOL CHEM, MAY 3 1996, 271 (18) P10433-6, XP002051361 UNITED STATES see the whole document ---	1-13, 21-28
A	MALO MS ET AL: "Targeted gene walking by low stringency polymerase chain reaction: assignment of a putative human brain sodium channel gene (SCN3A) to chromosome 2q24-31." PROC NATL ACAD SCI U S A, APR 12 1994, 91 (8) P2975-9, XP002051362 UNITED STATES see the whole document ---	1-13, 21-28
P,X	ISHIBASHI K ET AL: "Molecular cloning of a DEG/ENaC sodium channel cDNA from human testis." BIOCHEM BIOPHYS RES COMMUN, APR 17 1998, 245 (2) P589-93, XP002080466 UNITED STATES see the whole document ---	1-16, 21-28
T	WALDMANN R ET AL: "Molecular cloning of a non-inactivating proton-gated Na <sup>+</sup> channel specific for sensory neurons." J BIOL CHEM, AUG 22 1997, 272 (34) P20975-8, XP002080467 UNITED STATES see the whole document -----	1-6, 21-28

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/02884

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18,20  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Claims 18 and 20, relating to an agonist, antagonist to the polypeptide of claim 10-12, could not be searched as its subject-matter was insufficiently disclosed.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/02884

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9701577 A	16-01-1997	AU 6234596 A	30-01-1997
		CA 2221294 A	16-01-1997
		EP 0835261 A	15-04-1998
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